NADPH:cytochrome *P*-450 reductase in olfactory epithelium

Relevance to cytochrome *P*-450-dependent reactions

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1. The presence of a very active cytochrome *P*-450-dependent drug-metabolizing system in the olfactory epithelium has been confirmed by using 7-ethoxycoumarin, 7-ethoxyresorufin, hexobarbitone and aniline as substrates, and the reasons for the marked activity of the cytochrome *P*-450 in this tissue have been investigated. 2. The spectral interaction of hexobarbitone and aniline with hepatic and olfactory microsomes has been examined. By this criterion there was no evidence for marked differences in the spin state of the cytochromes of the two tissues, or for the olfactory epithelium containing a greater amount of cytochrome capable of binding hexobarbitone, a very actively metabolized substrate. 3. Rates of NADPH and NADH:cytochrome *c* reductase activity were found to be higher in the olfactory epithelium than in the liver, and direct evidence was obtained for a greater amount of the NADPH-dependent flavoprotein in the olfactory microsomes. 4. Investigation of male rats and male and female mice, as well as male hamsters, demonstrated that, in all cases, the cytochrome *P*-450 levels of the olfactory epithelium were lower than those of the liver, while the 7-ethoxycoumarin de-ethylase and NADPH:cytochrome *c* reductase activities were higher. A correlation was found between 7-ethoxycoumarin de-ethylase and NADPH:cytochrome *c* reductase activities for both tissues in all species examined. 5. The ratio of reductase to cytochrome *P*-450 was found to be considerably higher in the olfactory epithelium (1:2–1:3) than in the liver (1:11–1:15), regardless of the species examined, suggesting that facilitated electron flow may contribute significantly to the cytochrome *P*-450 catalytic turnover in the olfactory tissue.

INTRODUCTION

It has recently been demonstrated that the olfactory epithelium of the nasal cavity of a number of species contains cytochrome *P*-450 (Hadley & Dahl, 1982, 1983), and is capable of metabolizing many compounds including nitrosamines (Brittebo & Tjalve, 1981, 1982; Brittebo *et al.*, 1981), carbon tetrachloride (Tjalve & Lofberg, 1983), carbon disulphide (Bergman, 1984), benzo[*a*]pyrene (Bond, 1983) and progesterone (Brittebo, 1982). Both *in vivo* and in isolated tissue preparations the olfactory epithelium is very active in drug metabolism, in many cases more active than the liver (Tjalve & Lofberg, 1983; Brittebo & Tjalve, 1981; Hadley & Dahl, 1982; Dahl *et al.*, 1982). The reasons for this unusually high drug-metabolizing activity are not yet known.

Multiple forms of cytochrome *P*-450 have been characterized in liver microsomes (Lu & West, 1980), and in this tissue differences in drug metabolism are often due to differences in the composition and proportions of the various cytochrome *P*-450 species. These differences may be either genetically determined or experimentally produced by treatment of animals with inducing agents. The isoenzyme profile of the olfactory cytochrome *P*-450 has not yet been characterized, and may account for the high rates of olfactory drug metabolism.

Another factor that has been shown to affect rates of hepatic drug metabolism is microsomal electron transport. Under certain conditions the provision of either the first or the second electron to cytochrome *P*-450 has been implicated as rate-limiting in the catalytic cycle (Miwa *et al.*, 1978; Schenkman & Cinti, 1970; Taniguchi *et al.*,

1984). Furthermore, manipulation of the NADPH:cytochrome *P*-450 reductase to cytochrome *P*-450 ratio, either in reconstituted systems or by incorporation of flavoprotein into microsomal membranes, has been found to cause a progressive increase in the metabolism of a variety of substrates as the ratio increases from the *in vivo* value of 1:10–25 to a value of 1:1 (Miwa *et al.*, 1979; Miwa & Lu, 1984; Kitada *et al.*, 1979).

In this work we have confirmed the presence of a very active cytochrome *P*-450 in microsomes prepared from olfactory epithelium. We have examined the electrophoretic and substrate-binding properties of hepatic and olfactory cytochrome *P*-450, and also investigated components of the associated electron transport systems. We report that microsomes from olfactory tissue contain significantly more NADPH:cytochrome *P*-450 reductase than do those from liver, resulting in an unusually high reductase:cytochrome *P*-450 ratio in the former tissue. Comparison of the two tissues across different species has shown that a high reductase:cytochrome *P*-450 ratio is an important characteristic property of the olfactory epithelium, and we feel that it may play an important role in the high drug-metabolizing activity of this tissue.

MATERIALS AND METHODS

Materials

Horse heart cytochrome *c*, NADP, NADPH, 7-ethoxycoumarin, lumiflavin, FMN, luciferase, sodium cholate, 2'-AMP and bovine serum albumin were all obtained from Sigma Chemical Co. Glucose 6-phosphate

and glucose-6-phosphate dehydrogenase were purchased from Boehringer Mannheim, decanel, 7-hydroxycoumarin and resorufin from Aldrich Chemical Co., 7-ethoxyresorufin from Pierce, DEAE-Sephacel from Pharmacia Fine Chemicals and 2',5'-ADP agarose from PL Biochemicals. Other chemicals used were of the highest quality available commercially.

Preparation of microsomes

MF1 mice, Porton rats and Syrian hamsters were between 6 and 8 weeks old and were allowed food and water *ad libitum*. To prepare the olfactory tissue the animals were decapitated and the ethmoturbinates removed as described by Hadley & Dahl (1982). The tissue was homogenized and olfactory microsomes were prepared according to the method of Hadley & Dahl (1982). The final pellet was resuspended in 0.1 M sodium/potassium phosphate buffer, pH 7.4, containing 1 mM-EDTA unless stated otherwise. Livers were homogenized in sufficient 0.25 M-sucrose to give a 20% (w/v) homogenate, and microsomes prepared by differential ultracentrifugation. Final pellets were resuspended as the olfactory microsomes.

Spectral and metabolic assays

Cytochromes *P*-450 and *b*₅ were determined by the method of Omura & Sato (1964). Binding spectra for hexobarbitone and aniline were measured at 22 °C by the method of Schenkman *et al.* (1967).

7-Ethoxycoumarin and 7-ethoxyresorufin de-ethylase activities were measured according to the methods of Wolf *et al.* (1983) and Lake & Paine (1983) respectively, except that the incubation volumes were 1 ml and the substrate concentration 0.1 mM and 1 μ M respectively. An NADPH-generating system (De Matteis & Sparks, 1973) was used for both assays and incubations were at 37 °C with shaking (100 double oscillations/min) with protein concentration and time of incubation within the linear range. Fluorescence was measured in a Perkin-Elmer LS-5 luminescence spectrometer.

Hexobarbitone oxidase was measured by the modified (Copper & Brodie, 1955) method of Brodie *et al.* (1953).

Aniline hydroxylase was measured by the method of Schenkman *et al.* (1967), the aniline being redistilled prior to use.

Flavoprotein assays

NADPH and NADH:cytochrome *c* reductase activities were measured at 28 °C according to the method of Slater & Sawyer (1969) and Mihara & Sato (1972) respectively.

FMN content was determined by a bacterial luciferase reaction and the light production measured with an LKB Luminometer 1250. The method of Chapelle & Picciolo (1979) was followed for extraction and reduction of FMN, while the conditions of the luciferase assay were according to Stanley (1978). As the tissue extract was found to inhibit light production of an authentic FMN solution an internal standard was routinely added to the extracted samples prior to assay. Correction for extraction efficiency was carried out in a similar way.

Preparation of NADPH:cytochrome *P*-450 reductase

NADPH:cytochrome *P*-450 reductase was prepared from hamster liver essentially by the method of Yasukochi & Masters (1976) but with the following

modifications. FMN (2 μ M), phenylmethanesulphonyl fluoride (0.4%) and glycerol (20%) were included during solubilization and the supernatant fraction after centrifugation was applied to a DEAE-Sephacel column (2.6 cm $\times$ 30.0 cm) equilibrated with 0.1 mM-Tris buffer, pH 7.7, containing 20% glycerol, 0.1 mM-dithiothreitol, 0.1 mM-EDTA, 2 μ M-FMN, 0.8% Renex 690 and 0.1% sodium cholate. Elution from the 2',5'-ADP-agarose column (1.0 cm $\times$ 3.0 cm) was stepwise with 1 mM-2'-AMP followed by 5 mM-2'-AMP and 5 mM-2'-AMP plus 1% Renex 690. The pooled fractions were dialysed for 48 h against 50 mM-potassium phosphate buffer, pH 7.25, containing 20% glycerol, and concentrated in an Amicon concentrator.

Other assays

Cytochrome oxidase activity was measured at 30 °C with a Hansatech oxygen electrode. The sample was diluted in 82.5 mM-sodium/potassium phosphate buffer, pH 7.2, and placed in the cell together with the following components: *NNN'*-tetramethyl-*p*-phenylenediamine (0.3 mM), ascorbate, (20 mM) and EDTA (1 mM) in a 1 ml volume. The background rate of oxygen uptake was monitored and then cytochrome *c* (final concentration 0.32 mg/ml) added. Oxygen utilization was calculated on the basis that buffer at 30 °C contains 0.23 μ mol of O₂/ml.

SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970) and the gels were stained with Coomassie Blue. Protein concentrations were estimated by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Kinetic data for the determination of *K*_m, *K*_s and maximum ΔA values was analysed by least squares linear regression. The data for the correlation between 7-ethoxycoumarin de-ethylase activity and either cytochrome *P*-450 content or reductase activity was treated similarly.

RESULTS

Cytochrome *P*-450 content activity

We have demonstrated a typical cytochrome *P*-450 difference spectrum in microsomes prepared from olfactory epithelium of male hamsters, and found that, expressed per mg of microsomal protein, this tissue contained approximately one-half of the cytochrome *P*-450 of hepatic microsomes (Table 1). However, the metabolism of four widely used cytochrome *P*-450 substrates, 7-ethoxycoumarin, 7-ethoxyresorufin, hexobarbitone and aniline was, on a per mg of protein basis, 7, 3, 33 and 5 times more rapid in the olfactory epithelium than the liver (Table 1). When expressed per nmol of cytochrome *P*-450 the metabolism of these substrates in the olfactory epithelium was 15, 5, 66 and 10-fold greater, respectively, than in the liver.

To determine whether or not cytochrome *P*-450 was involved in this active drug metabolism we investigated the effect of various incubation conditions on 7-ethoxycoumarin de-ethylation. In olfactory epithelium omission of NADPH reduced activity to less than 1% of control values, while 10 mM-KCN or incubation in an atmosphere of CO/O₂ (4:1) reduced activity to 12% and 11% of controls, respectively. In the liver these values were 1%, 43% and 33%. Thus the drug-metabolizing

Table 1. Cytochrome P-450 and drug metabolism in microsomes isolated from liver and olfactory epithelium of male hamsters

Experimental conditions were as described in the Materials and methods section. Hexobarbitone oxidase results are means of two observations (individual values in parentheses), each on tissues pooled from eight animals. Other results are means $\pm$ S.D. of at least three separate observations, each obtained with tissues pooled from at least two animals.

	Liver	Olfactory epithelium
Cytochrome P-450 (nmol/mg of protein)	1.15 $\pm$ 0.19	0.58 $\pm$ 0.14†
7-Ethoxycoumarin de-ethylase (nmol/min per mg of protein)	3.74 $\pm$ 0.58	27.10 $\pm$ 11.9†
(nmol/min per nmol of cytochrome P-450)*	3.22	46.72
7-ethoxyresorufin de-ethylase (nmol/min per mg of protein)	0.21 $\pm$ 0.05	0.56 $\pm$ 0.10†
(nmol/min per nmol of cytochrome P-450)*	0.18	0.97
Hexobarbitone oxidase (nmol/h per mg of protein)	1.4 (1.6, 1.1)	45.9 (43.0, 48.8)
(nmol/h per nmol of cytochrome P-450)*	1.2	79.1
Aniline hydroxylase (nmol/min per mg of protein)	0.95 $\pm$ 0.15	4.67 $\pm$ 0.73†
(nmol/min per nmol of cytochrome P-450)*	0.83	8.05

* Calculated using mean cytochrome P-450 levels shown in the Table.

† $P < 0.01$ when compared with corresponding value obtained with liver microsomes.

Table 2. Spectral interaction of hexobarbitone and aniline with hepatic and olfactory microsomes of male hamsters

Experimental conditions were as in the Materials and methods section. Results are either means $\pm$ S.D. of three separate observations or means of two observations with individual values in parentheses. Each observation was obtained with tissue pooled from 10–15 animals.

Compound	Tissue	K_s (mM)	Max. ΔA /nmol of cytochrome P-450
Hexobarbitone	Liver	0.061 (0.062, 0.059)	0.015 (0.013, 0.016)
	Olfactory epithelium	0.032 (0.035, 0.029)	0.022 (0.019, 0.025)
Aniline (a) 0.088–0.264 mM	Liver	0.24 $\pm$ 0.07	0.016 $\pm$ 0.003
	Olfactory epithelium	0.47 (0.31, 0.63)	0.020 (0.018, 0.021)
	Liver	1.01 $\pm$ 0.05	0.037 $\pm$ 0.003
	Olfactory epithelium	1.55 $\pm$ 0.31	0.044 $\pm$ 0.007

activity of the olfactory epithelium appears to be cytochrome P-450-dependent.

The kinetics of 7-ethoxycoumarin de-ethylation in hamster hepatic and olfactory microsomes were examined by double-reciprocal plots of enzymatic activity at increasing substrate concentration. In the liver there appeared to be two distinct phases with corresponding K_m values of $4.5 \pm 0.4 \mu\text{M}$ and $53.3 \pm 14.8 \mu\text{M}$, possibly indicating two catalytic sites for 7-ethoxycoumarin metabolism varying significantly in affinity for the substrate. In the olfactory epithelium only one phase was noted, with a K_m value of $7.1 \pm 2.9 \text{ mM}$.

Spectral studies

Addition of hexobarbitone to either hepatic or olfactory microsomes resulted in a typical type I spectral change with an absorption maximum at 390 nm and minimum at 420 nm. Both the affinity for hexobarbitone and the maximum ΔA per nmol of cytochrome P-450 were marginally higher in the olfactory epithelium than the liver (Table 2). Addition of aniline to hepatic microsomes resulted in an asymmetrical type II spectral change and double-reciprocal plots of ΔA values at increasing substrate concentrations showed the binding

Table 3. Components of the microsomal electron transport system of liver and olfactory epithelium of male hamsters

Experimental conditions were as described in the Materials and methods section. Results are means $\pm$ S.D. for at least three separate observations on tissue pooled from more than one animal.

Component	Liver	Olfactory epithelium
Cytochrome b_5 (nmol/mg of protein)	0.60 ± 0.06	0.66 ± 0.04
NADPH:cytochrome c reductase (nmol of cytochrome c reduced/min per mg of protein)	116 ± 14	$372 \pm 46^*$
NADPH:cytochrome b_5 reductase (nmol of cytochrome c reduced/min per mg of protein)	1119 ± 141	$2056 \pm 134^*$

* $P < 0.01$, when compared with corresponding value obtained with liver microsomes.

Table 4. NADPH:cytochrome c reductase activity and FMN content of hepatic and olfactory microsomes of male hamsters

Experimental conditions were as described in the Materials and methods section. Results are means $\pm$ S.D. of three separate observations, each on pools of 11 and 12 animals.

Tissue	NADPH:cytochrome c reductase (nmol of cytochrome c reduced/min per mg of protein)	FMN (pmol/mg of protein)	Specific activity of reductase (nmol of cytochrome c reduced/min per nmol of FMN)
Liver	160 ± 19	102 ± 12	1569
Olfactory epithelium	443 ± 108	298 ± 86	1487

to be biphasic. The binding of aniline to olfactory microsomes was also biphasic, but the resultant spectral change was symmetrical. Calculation of the K_s and maximum ΔA per nmol of cytochrome $P-450$ for both phases showed that, while the K_s values in the olfactory epithelium were slightly higher than those in the liver, there were no significant differences in the maximum ΔA values (Table 2). Saturation of the microsomes with hexobarbitone prior to the addition of aniline abolished the asymmetry of the hepatic spectral change while having no effect on the shape of the olfactory spectrum; the maximum ΔA was increased in both tissues.

NADPH:cytochrome $P-450$ reductase and other components of the microsomal electron transport system

Although differences were found between olfactory epithelium and liver in both kinetic parameters and substrate binding, these were probably not sufficient to account for the vast differences between the two tissues in drug-metabolizing activity. When the amount or activity of the various components of the microsomal electron transport system were investigated in hamster liver and olfactory epithelium (Table 3), no difference in the cytochrome b_5 content was found. However, the activity of both the NADPH- and NADH-dependent flavoproteins (using cytochrome c as electron acceptor) was higher in the olfactory epithelium than the liver, by factors of 3.3 and 1.9 respectively.

In order to establish whether the amount, as well as the activity, of NADPH:cytochrome c reductase was increased in olfactory microsomes, the microsomal content of FMN was measured. NADPH:cytochrome $P-450$

reductase is the only microsomal flavoprotein containing FMN as its prosthetic group; NADH:cytochrome b_5 reductase and the flavin-containing mono-oxygenase contain only FAD (Mihara & Sato, 1978; Ziegler & Poulsen, 1978). However, since mitochondrial NADH dehydrogenase contains FMN, mitochondrial contamination was determined (using cytochrome oxidase as marker) and corrected for. From Table 4 it can be seen that the olfactory epithelium not only had greater NADPH:cytochrome c reductase activity than the liver, but also more FMN, and that the specific activity of the flavoprotein was similar in the two tissues.

Confirmation that the amount of NADPH-dependent flavoprotein was in fact greater in olfactory microsomes was obtained by gel electrophoresis of olfactory and hepatic microsomes alongside the purified flavoprotein (Fig. 1). It will be noticed that the intensity of the band corresponding to the olfactory reductase suggests an increase greater than that seen by activity or FMN content. This suggests that some of the flavoprotein may be present in a functionally inactive form, as reported for cytochrome b_5 as well as NADPH:cytochrome $P-450$ reductase by Shawver *et al.* (1984). The gel electrophoresis also showed marked differences between the two tissues in the M_r 45000–60000 region where cytochrome $P-450$ isoenzymes would be expected to run (Fig. 1). Whereas the liver had three or four major bands in this area there was a single, predominant band and one or two smaller ones in the olfactory epithelium. The major olfactory protein may not correspond in M_r to any of the hepatic proteins, but more work is required to establish this with certainty.

Fig. 1. SDS/polyacrylamide-gel electrophoresis of hamster liver and olfactory microsomes and purified NADPH:cytochrome *P*-450 reductase

Experimental conditions were as described in the Materials and methods section. Lane (a), M_r markers; lane (b), olfactory epithelium (20 μ g of microsomal protein); lane (c) purified reductase (2 μ g of protein); lane (d), liver (20 μ g of microsomal protein).

Components of the drug-metabolizing system of liver and olfactory epithelium of different species

Cytochrome *P*-450 levels, NADPH:cytochrome *c* reductase and 7-ethoxycoumarin de-ethylase activities were measured in hepatic and olfactory microsomes of male rats and hamsters and male and female mice (Table 5). In all cases the liver had 2–4 times more cytochrome *P*-450 than the olfactory epithelium, while the latter tissue had considerably greater reductase and de-ethylase activities. 7-Ethoxycoumarin de-ethylase activity was linearly related to cytochrome *P*-450 content in both liver and olfactory epithelium (Fig. 2a), but the two tissues formed two discrete lines of correlation, suggesting the involvement of additional factors. When 7-ethoxycoumarin de-ethylase activity was related to NADPH:cytochrome *c* reductase activity a single line of correlation was found, encompassing results from both tissues. (Fig. 2b)

Assuming a specific activity of 1528 nmol of cytochrome *c* reduced/min per nmol of FMN (the mean of the hamster hepatic and olfactory specific activities) for all species, sexes and tissues the NADPH:cytochrome *P*-450 reductase to cytochrome *P*-450 ratio was calculated for the various samples (Table 5). It was found that in the liver the ratio varied from 1:11 to 1:15, while in the olfactory epithelium it was 1:2 or 1:3 in every case. Therefore, when compared with the liver, both increased drug metabolism and a high reductase cytochrome *P*-450 ratio appear to be characteristic properties of the olfactory epithelium.

DISCUSSION

These results confirm the presence of cytochrome *P*-450 in the olfactory epithelium of a number of rodent species, and show that, in the hamster, this tissue is more

Table 5. Cytochrome *P*-450 content and NADPH:cytochrome *c* reductase and 7-ethoxycoumarin de-ethylase activities of hepatic and olfactory microsomes of different species and sexes

Experimental conditions were as described in the Materials and methods section. Results are means $\pm$ S.D. of at least three separate observations, each on tissue pooled from one or more animals.

Species	Sex	Tissue	Cytochrome <i>P</i> -450 (nmol/mg) (A)	7-Ethoxycoumarin de-ethylase (nmol/min per mg of protein)	NADPH:cytochrome reductase <i>c</i> (nmol of cytochrome <i>c</i> reduced/min per mg of protein)	NADPH:cytochrome <i>P</i> -450 reductase (nmol/mg of protein)* (B)	Ratio B:A
Hamster	M	Liver	1.15 $\pm$ 0.19	3.70 $\pm$ 0.6	116 $\pm$ 14	0.076	1:15
		Olfactory epithelium	0.58 $\pm$ 0.14	27.10 $\pm$ 11.9	372 $\pm$ 46	0.243	1:2
Rat	M	Liver	0.53 $\pm$ 0.06	0.76 $\pm$ 0.16	68 $\pm$ 12	0.045	1:12
		Olfactory epithelium	0.19 $\pm$ 0.06	3.40 $\pm$ 1.66	100 $\pm$ 38	0.065	1:3
Mouse	M	Liver	0.74 $\pm$ 0.11	1.03 $\pm$ 0.14	84 $\pm$ 12	0.055	1:13
		Olfactory epithelium	0.29 $\pm$ 0.04	17.89 $\pm$ 3.00	231 $\pm$ 26	0.151	1:2
Mouse	F	Liver	0.75 $\pm$ 0.06	1.32 $\pm$ 0.10	108 $\pm$ 7	0.071	1:11
		Olfactory epithelium	0.19 $\pm$ 0.08	6.24 $\pm$ 1.50	152 $\pm$ 24	0.099	1:2

* Calculated from mean specific activity of hamster liver and olfactory epithelium reductase [1528 nmol of cytochrome *c* reduced/min per nmol of FMN (Table 4)].

Fig. 2. Relationship between 7-ethoxycoumarin de-ethylase activity and (a) cytochrome *P*-450 content or (b) NADPH:cytochrome *c* reductase activity in liver and olfactory epithelium

Experimental conditions were as described in the Materials and methods section. Open symbols represent liver, closed ones olfactory epithelium. ■, □, male hamster; ▲, △, male rat; ●, ○, male mouse; ▼, ▽, female mouse. Results are from Table 5.

active than the liver in the cytochrome *P*-450-dependent metabolism of four compounds. Although all four substrates were metabolized more rapidly in the olfactory epithelium the ratios of nasal to liver activity varied considerably (15:1, 5:1, 66:1 and 10:1 for 7-ethoxycoumarin, 7-ethoxyresorufin, hexobarbitone and aniline, respectively). These high rates of olfactory drug metabolism might result from either intrinsic, quantitative or qualitative differences between the olfactory and hepatic cytochromes *P*-450, or from other factors, extrinsic to the haemoprotein. Each of these possibilities will be considered in turn.

Attempted characterization of olfactory cytochrome *P*-450

We have found some evidence for differences between the cytochrome *P*-450 species of the liver and olfactory epithelium. (1) The electrophoretic profiles of hepatic and olfactory microsomes differ markedly in the M_r 45000–60000 region, with the olfactory epithelium showing only one major band in this area. However, since none of the bands have been positively identified as cytochrome *P*-450 no definite conclusions can be drawn without further investigation. (2) The kinetic parameters for 7-ethoxycoumarin metabolism are different in the two tissues. Olfactory 7-ethoxycoumarin de-ethylase activity appeared to involve a single catalytic species (with high affinity for the substrate) while there was evidence for more than one catalytic site in the liver (a low-affinity as well as a high-affinity site). This is compatible with previous findings that the drug can be metabolized by more than one hepatic cytochrome *P*-450 species (Lu & West, 1980; Norman *et al.*, 1978; Ryan *et al.*, 1979).

The change induced by hexobarbitone in the spectrum of oxidized cytochrome *P*-450 was studied in both tissues in an attempt to characterize the binding capacity of the

haemoproteins and, more specifically, to titrate that portion of the total cytochrome *P*-450 which can accept the drug as a substrate. Only marginal differences in both K_s and maximum ΔA were found between the two tissues. The type I spectral change indicates transition of the haem iron from a low-spin to a high-spin state, so if the haem of the olfactory cytochrome were to exist in a predominantly high-spin form, the addition of hexobarbitone would not elicit a spectral change of great magnitude however extensive the binding. Type II compounds, such as aniline, effect the reverse transition, namely high-spin to low-spin. Thus we argued that, if the olfactory cytochrome *P*-450 was predominantly high-spin, then the addition of aniline should result in an unusually large spectral change. This was not found to be the case; as with hexobarbitone there were no significant differences between maximum ΔA values per nmol of cytochrome *P*-450 of the liver and olfactory epithelium. This suggests that the results obtained with hexobarbitone reflect a similar extent of binding in the two tissues, despite the 66-fold difference in metabolism of this substrate.

The asymmetry of the hepatic spectral change induced by aniline requires some comment. This has been ascribed to a composite type I and type II change (Schenkman, 1970), with aniline binding as a substrate and as a ligand respectively. Inclusion of hexobarbitone saturates the type I binding sites so that the resultant spectral change is solely of a type II nature (Schenkman, 1970). The asymmetry of the aniline binding spectrum in hamster liver microsomes, and the alterations seen after saturation with hexobarbitone, were similar to those reported by Schenkman (1970). However, the olfactory spectral change was not asymmetrical and hexobarbitone had little effect, suggesting that the olfactory cytochrome *P*-450 contains relatively few sites for aniline to bind in a type I manner, that is as a substrate for mono-

oxygenation. Despite this, the aniline hydroxylase activity of the olfactory epithelium was 10-fold greater than that of the liver.

Thus, with both these substrates no evidence could be found of increased capacity for binding by cytochrome P-450 in the olfactory epithelium as compared with the liver. In spite of this there were very marked differences in metabolizing activity. So, by this criterion no major quantitative difference in the amount of cytochrome P-450 capable of metabolizing hexobarbitone and aniline was apparent between the two tissues. Therefore alternative explanations for the very high metabolic activity of the olfactory cytochromes must be considered. They may possess unusual catalytic properties (a possibility we have not yet explored) or some other factor may be involved, for instance the satellite components of the electron transport system.

Components of the electron transport system

An important finding of this present work has been the increased activity in the olfactory epithelium of the two flavoproteins that donate electrons to cytochrome P-450, particularly of NADPH:cytochrome P-450 reductase. The amount of the NADPH-dependent reductase has also been shown to be increased by direct estimation of the FMN content of the microsomes and by gel electrophoresis of microsomal proteins alongside the purified reductase.

A consequence of the high concentration of NADPH:cytochrome P-450 reductase is that, assuming that the two proteins are part of the same membrane system, the reductase to cytochrome P-450 ratio of the olfactory epithelium is significantly greater than that of the liver (1:2, compared with 1:15 in hamsters). Previous work in reconstituted systems has shown that rates of drug metabolism increase as the ratio of NADPH:cytochrome P-450 reductase to cytochrome P-450 increases, with an optimum ratio of 1:1 (Miwa & Lu, 1984; Miwa *et al.*, 1979). Furthermore, addition of purified reductase to liver microsomes has been shown to increase rates of metabolism of a number of substrates, including 7-ethoxycoumarin (Kitada *et al.*, 1979; Miwa *et al.*, 1978). All these were artificial situations, but the evidence discussed below suggests that the same may apply to the intact membranes of the olfactory epithelium.

When different species and sexes were investigated for their cytochrome P-450 levels, 7-ethoxycoumarin de-ethylase and NADPH:cytochrome *c* reductase activities, the following became apparent. (1) High rates of NADPH:cytochrome *c* reductase activity were not only seen with hamster olfactory epithelium, but appeared to be a general property of this tissue. (2) 7-Ethoxycoumarin de-ethylase was, as would be expected, linearly related to cytochrome P-450 content in both the liver and the olfactory epithelium. However, the two tissues formed two discrete lines of correlation, suggesting the involvement of one (or more) additional, tissue-specific factor(s). (3) The reductase:cytochrome P-450 ratio was similar in all the liver samples studied and similar, but different to the liver, in all the olfactory samples.

This latter finding suggests that the bulk of the olfactory reductase is located in the same membrane system as cytochrome P-450 as it would seem unlikely that a constant ratio of the two components would be found across different species and sexes if they were not part of the same integrated system. The increased

reductase:cytochrome P-450 ratio of the olfactory epithelium also implies that the organization within the endoplasmic reticulum of components of the drug-metabolizing system of this tissue differs from that of the liver. This might well affect rates of drug metabolism, and we would like to suggest that the high olfactory reductase:cytochrome P-450 ratio may be one at least of the tissue-specific factors mentioned in (2) above, and contributes to the active drug metabolism of the olfactory epithelium.

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